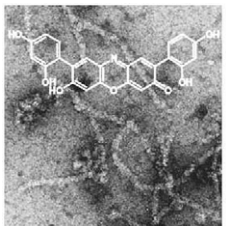
amyloid fibrils cause neuronal dysfunction in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Biochemical and cell biological studies indicate that amyloid formation pathways can be manipulated with small molecules. This suggests that stimulation of amyloid polymerization with small molecules might reduce the prevalence of transient, toxic aggregation intermediates. We have recently demonstrated the acceleration of alpha-synuclein and Abeta fibrillogenesis through the action of the orcein-related small molecule, which leads to a decrease in toxicity neuronal cell models.



These results support the hypothesis that small, diffusible pre-fibrillar amyloid species rather than mature fibrillar aggregates are toxic for mammalian cells. They also suggest that compound-mediated acceleration of amyloidogenesis might be a promising therapeutic strategy for amyloid diseases.

2314-Pos Board B84
Alzheimer's Disease at 30? Is that Possible?
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Amyloid diseases, such as Alzheimer's disease, are neurodegenerative disorders that have been introduced by protein misfoldings into amyloid fibrils. The Amyloid Precursor Protein irregularly cleaves the β-amyloid (Aβ) peptide, causing protein misfoldings to aggregate to form the hallmark plaques. For both Aβ(1-40) and Aβ(1-42), a tendency of fibril formation has demonstrated to self-assemble from a non-toxic monomer state to a lethal fibrillar state. Prior research focused only on the 16-21 region of the peptide, however, it is equally important to examine the hair-pin region without the presence of residues 16-21. In order to study the effect on fibril formation without this ''KLVFFA'' region, the 22-35 sequence was chosen . The Italian (E22K) and Arctic (E22G) point mutations lead to changes in time of fibril formation as well as solubility and toxicity of fibrils. The single-point mutations are believed to promote early onset of AD compared to the wild type (WT), prematurely producing clinical and neuropathological features which are unchanged from those of late onset AD. The use of Attenuated Total Reflection Infrared Spectroscopy, ATR-IR, and Ultraviolet Visible Spectroscopy, UV-Vis, on the 22-35 sequence confirmed the formation of structures synonymous with toxic beta sheets. Using Congo Red dye, which binds pentamerically to beta sheet fibrils, secondary structures have been confirmed.

2315-Pos Board B85
Peptides - Beta-Sheet Folding from Hairpins to Aggregates
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Selected examples of turn sequence and hydrophobic contact stabilized β-hairpin peptides were previously studied using ECD, fluorescence, IR and VCD spectroscopies to assess stability of β-hairpin formation. Extending this, two three-stranded β-sheet peptides, based on modified Trpzip sequences, using D-Pro-Gly (B3pG) or Asn-Gly (B3GN) turn sequences gave ECD spectra reflecting cross-strand Trp-Tyr aromatic interactions, and indicated both were partially multi-stranded. Both showed initial IR spectra at low temperatures indicative of extended β-sheet structure that were more characteristic of an aggregate than a small oligomer structure. Thermal variation of their IR spectra gave strikingly different behaviors. B3pG reversibly unfolded from aggregated β-like structure at low temperature to disordered at high temperature. B3GN formed aggregates at low temperature, became disordered with heating, but upon re-cooling gave typical soluble β-sheet peptide spectra, which was could be reversibly unfolded. ThT binding to dilute B3GN, but not B3pG, caused a fluorescence enhancement, consistent with fibril formation. These results suggest that turn sequence mutation leads to different micro- and macro-structures, resulting in tuning their structurally related properties. Modifying the sequences to reduce hydrophobicity (aromatic residues) but incorporate Aib-Gly turns gave partially folded peptides with reversible folding but less stability. Taking another view of β-sheet based peptide aggregation, we studied fibril formation in glutamic acid oligomers at low pH (β2 form). We had shown that poly-Glu IR is relatively insensitive to mixing of D and L isomers, which causes a loss of long range order (fibrilization), as seen in EM, but VCD is hypersentive to this change, providing a new way of detection of long range ordering (chirality) in fibril formation as opposed to aggregate forms. The oligo-Glu peptide models add isotopic labeling to provide new insight into the structure of the fibrils formed.

2316-Pos Board B86
Exploring the Effect of Mutations on the Conformational Landscape of Amyloidogenic Antibody Fragments
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Antibodies are composed of heavy chains and light chains. In Light Chain Amyloidosis (AL), antibody-secreting cells export only the light chains. These light chains are prone to misfold, forming amyloid fibers, which get deposited in various organs, leading to organ disfunction and patient death. The transition from the native state to the amyloid fiber requires partial or total denaturation. The structures of monomeric precursors to the oligomeric nuclei, or of those assembled at the ends of the fibers, are unknown. There is an inverse correlation between the stability of the native state and the speed of fiber formation, suggesting that lower stability allows the population of ''excited states'' of the native ensemble, some of which could be amyloid fiber precursors. Starting from the crystal structure of a variable light chain domain belonging to class 6a (one of the most common in clinical cases of AL), we generated point mutants that eliminate charges (R24G and D52A) or a proline (P7S). These mutants destabilize the native state, and speed up fiber formation (for R24G and P7S). We carried out MD simulations at three temperatures (298, 398 and 498K), to explore the effect of these mutations on the conformational landscape. We found many metastable unfolding intermediates, which have eluded experimental detection because their fluorescence is indistinguishable from that of the native state. A common early unfolding intermediate exposes strand D, which has a high potential for fiber formation according to ZipperDB. Those variants with a higher speed of fiber formation expose this area with greater frequency.
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2317-Pos Board B87
Monomeric Amyloid β Proteins in Reverse Micelles are in Folded β Sheet Structure
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Amyloid beta (Aβ) proteins aggregate to form insoluble fibrils in the brains of persons with Alzheimer's disease (AD). X-ray diffraction studies reveal cross-β structure in these fibrils, while solid state NMR studies indicate that this structure consists of in-register parallel β-sheets. Proteins with these characteristics accumulate in over a dozen different diseases, which have come to be known as protein misfolding diseases. The trigger that induces the misfolding of Aβ proteins in AD is unknown, and neither is the mechanism by which monomeric Aβ proteins add to the growing end of a fibril. In order to characterize the structure of Aβ proteins under conditions in which they are monomeric but not aggregated, we have encapsulated the 40-residue form of the Aβ protein (Aβ40) into reverse micelles formed from sodium bis (2-ethylhexyl) sulfosuccinate (AOT) and examined them with transmission FTIR spectroscopy. Several types of evidence indicate that the encapsulated proteins are monomeric, yet the spectra suggest that they have β-sheet secondary structure. The spectra of a polypeptide with the same amino acid composition as Aβ40 but with a scrambled sequence show a random-coil structure. These results suggest that Aβ40 is capable of forming both antiparallel β structures in a reverse micelle, and parallel β structure in an amyloid fibril. We speculate that antiparallel β structure may help pre-organize the protein for adding onto the end of a growing fibril.

2318-Pos Board B88
Eating and Sleeping, Is this a Cure for Alzheimer's Disease?
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The accumulation of misfolded amyloid proteins has proven to be the common link between neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's disease. According to previous studies done by numerous

researchers, the diffusible beta sheet intermediates are in fact the most critical step in the prevention of Alzheimer's disease (AD) due to their high toxicity levels and function as precursors to amyloid betaproteins. Research has been focused on the amyloid beta (22-35) portion of the single point wild type mutation because of its high toxicity level during the progression of AD. Orthomolecular compounds, such as melatoninand curcumin, will be combined with the amyloid peptide in various concentrations and times to test their preventative effects on the amyloid fibrils due to their neurotoxicity. The hormone melatonin is naturally secreted by the body and can not only reduce AD patient symptoms of insomnia but it can also disrupt Aβ toxicity in the early stages of AD. Additionally, curcumin is the key component of the plant turmeric. Attenuated Total Reflectance Infrared Spectroscopy (ATR-IR) along with Ultraviolet visible spectroscopy (UV/Vis) and electron microscopy (EM) will be used in order to determine the structural confirmation and morphology on the pathway to fibril formation. This knowledge will help determine the specific mechanism used to destabilize the intermediate structures before they can form amyloid plaque and possibly lead to a cure and/or preventative.

2319-Pos Board B89

Diversity of Sequences Folding to Highly and Poorly Designable Structures

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Protein structures are evolutionarily more conserved than sequences, and sequences with very low sequence identity often share the same fold. This leads to the concept of protein designability. Elucidating the relationship between protein sequence and the three-dimensional structure that the sequence folds into is an important problem in computational structural biology.

45 protein chains (40-mer) from the PDB were analyzed. Hydrophobic-polar sequences were generated and contact energies calculated by threading each sequence onto Cα coarse-grained protein structures. The minimum energy structure for each sequence was identified and the number of sequences folding to each fold (designability) was obtained. Highly designable structures obtained were found to be popular structural motifs.

H/P mutational analysis of sequences folding to each conformation was performed. As designability increases, the total number of mutations was also found to increase. The sequences folding to the most designable structure (helix-turn-helix motif) were also analyzed. The degree of connectivity at each residue position correlates inversely with the degree of solvent exposure. The surface residues had fewer interactions compared to buried residues. Highly connected residues were also found to be more conserved than the other residue positions. i.e. the diversity of the sequences increases with designability; however, there are conserved positions.

Using tripeptide percentages of the most and least designable sequences, ten-fold cross-validation was performed and designable sequences were found to be distinguishable (accuracies $> 85\%$, AUC > 0.87). The same set of sequences was then used as a training set with a test set of real binary protein sequences. Designable sequences obtained mimic real protein sequences with accuracies of nearly 60%. Highly and poorly designable classes can be used to train machine learning algorithms to identify which real protein sequences are designable.

2320-Pos Board B90

Modeling CLIC2-RyR Interactions and the Effect of Disease Causing Mutation

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Our recent in silico studies have shown that chloride intracellular 2 (CLIC2) protein harbors a missense mutation in the H101Q position and gives significant changes in CLIC2's stability. In this work, we extend our investigation to include the plausible effects of the mutation on CLIC2-RyR (ryanodine receptor 1) interactions. For this purpose, we built a 3D model of the CLIC2-RyR complex by using ab-initio docking. The models are evaluated against electron microscopy data to assure that the binding interface of CLIC2-RyR is recovered. The models are then used to evaluate the role of electrostatics on CLIC2-RyR recognition by carrying out calculations with Delphi (compbio.clemson.edu/delphi.php). The results of electrostatics calculations indicate that charge complementarity plays an important role in the binding and that the disease-causing mutation affects the electrostatic component of the binding. By combining the outcome of electrostatic calculations with other in silico analyses, including solvent accessible surface area, docking predictions, and binding free energies, we were successfully able to gain insight into the effects of a missense mutation on the stability of CLIC2-RyR complex. The work is supported by NIH, NIGMS, grant number 1R01GM093937-01.

2321-Pos Board B91

Sampling of Random Amino Acid Mutations: Folding and Binding Stability of Coat Proteins in a Simple Virus

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It is recognized that protein-protein interactions are a vital component of protein evolution since it is thought that well over half of proteins exist as protein complexes. Previous studies have shown that most random amino acid substitutions destabilize protein folding (i.e., increase the folding free energy), however, no such study has been carried out for protein-protein binding. Thus, we used FoldX to estimate the free energy of folding and binding for coat proteins in a simple virus. Our results suggest that most random mutations destabilize protein folding, consistent with previous findings. However, by contrast, most random mutations stabilize protein-protein binding. In addition, natural selection appears to favor stabilizing folding rather than stabilizing binding for this virus. Finally, the temperature of the virus affects binding stability more strongly than folding stability.

2322-Pos Board B92

Hamiltonian Replica Exchange Simulations to Enhance Sampling for Protein Folding

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In order to enhance sampling in biomolecular simulation many efforts have focused on reducing the effective degrees of freedom and employing coarse-grained models. Structure-based or Go-models for protein folding are based on the energy landscape theory and the principle of minimal frustration have demonstrated good agreement with experimental measurements and are computationally sufficiently tractable to allow simulating large-scale structural transitions and provide sufficient sampling even of large-scale conformational transitions. Linking these minimal or coarse-grained models to physically motivated empirical force fields would enhance understanding of the underlying physical/chemical interactions.

Here, we present a novel approach combining an efficient coarse-grained native structure-based model with a physics based all-atom model. This approach targets the sampling problem that has plagued traditional simulation methods for decades while providing an energetically accurate description of conformational transitions. Our approach is based on Hamiltonian exchange, a variant of the Replica-exchange concept, by coupling the Hamiltonians that operate not on different temperatures, but on different levels of representation. Each level is occupied by a combination of the two mixed Hamiltonians $H\text{total}=\lambda H1+(1-\lambda)H2$. During simulation, one permits exchanges between neighboring levels. We observe frequent transitions between neighboring levels and an enhanced sampling efficiency for model proteins.

2323-Pos Board B93

Analysis of Amino Acid Specific Energy Contributions to Native Conformations in High-Resolution Protein Structures

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Because most proteins are marginally stable, each amino acid must contribute a near-optimal stabilization energy to the overall protein-structure. In this study we have analysed the specific energy contribution of each amino acid found in the native conformation of globular proteins. Using our free-energy forcefield PFF02 we devise a local energy measure on a per-amino acid basis and relax a population of 50 high resolution experimental globular protein structures on POEM@HOME with an evolutionary Monte Carlo energy minimization algorithm. The obtained set of relaxed structures is screened for locally stable segments, ruling out intrinsical disorder, and datasets of the energy ranges are assembled correlated with the position of the amino acid (interior, exterior, solvent-exposed).

POEM (Protein Optimization using Energy Methods) identifies the native conformation of the protein as the global minimum of the protein free-energy forcefield PFF02, which stabilized the native conformation of all 32 monomeric proteins (without cofactors) against all decoys in the Rosetta decoy set. In addition we could fold a set of 13 proteins with helical, sheet and mixed secondary structure from completely unfolded conformations to near-native conformations, to an average 2.87 Å resolution.

The simulations we have conducted, were run on the POEM@HOME (http://boinc.fzk.de) volunteer computing architecture using a multiple population evolutionary strategy, which explores the free-energy surface in many parallel Monte-Carlo random walks. Various distinct temperature populations are evolved to the global free-energy minimum by balancing energy improvement and population diversity.